Amendments to the Specification:

Please replace the paragraph beginning at line 34 of page 7 with the following amended paragraph:

(C) Analysis of p27 protein products in $p27^{+/+}$ and $p27^{-/-}$ mouse embryonic fibroblasts. Immunoprecipitates of p27 or whole cell lysates were subjected to SDS-PAGE and immuoblotted with the antibodies described to the left of the panels. Antibody abbreviations are: p27-carboxyl terminal specific antibody (αp27-CT), p27-amino terminal specific antibody (αp27-NT), CDK2 and CDK4 carboxyl specific antibodies (αCDK2 and αCDK4, respectively), rabbit anti mouse Ig (RAM). Markers and the migration of the Ig heavy chain are indicated. FIG. 1C-1 provides the result from immunoprecipitation with αp27-CT and detection with αp27-CT. FIG 1-C2 provides the result from detection with αp27-NT of total cell lysate from $p27^{+/+}$ and $p27^{-/-}$ mouse embryonic fibroblasts. FIG. 1C-3 provides the result from immuno-precipitation with αp27-CT and RAM and detection with αCDK2. FIG. 1-C4 provides the results from immunoprecipitation with αp27-CT and RAM and detection with αCDK4.

Please replace the paragraph beginning at line 11 of page 8 with the following amended paragraph:

(D) Δ51 protein is not able to inhibit G1-CDK activity. Lysates were prepared from Sf9 cells coinfected with the baculoviruses expressing cyclin and CDK subunits indicated above each graph. FIG. 1D-1 depicts the kinase activity results for lysates prepared from Sf9 cell coinfected with baculoviruses expressing cyclin E and CDK2. FIG. 1D-2 depicts the kinase activity results for lysates prepared from Sf9 cell coinfected with

baculoviruses expressing cyclin D2 and CDK4. Equal amounts (30 nM) of either p27 (black) or Δ51 (open) were added and kinase activity measured, relative to a control (hatched) where neither protein was added. The kinase activity was determined on a GST-Rb substrate and plotted as percentage of the control.

Please replace the paragraph beginning at line 37 of page 9 and continuing onto page 10 with the following amended paragraph:

(A) $p27^{-/-}$ mice have enlarged pituitaries which can be attributed to selective hyperplasia of the intermediate lobe. A composite picture comparing hemotoxylin and eosin stained pituitary sections ~~(top)~~ from an eleven week old $p27^{-/-}$ (~~left~~ FIG. 4A-1), 11 week old $p27^{-/-}$ (~~middle~~ FIG. 4A-2) and a 30 week old $p27^{-/-}$ (~~right~~ FIG. 4A-3) mouse. p, posterior lobe; I, intermediate lobe; a, anterior lobe. The intermediate lobe of 11 week old $p27^{-/-}$ mice is increased in size relative to the $p27^{+/+}$ controls with little change in cellular composition. In 30 week old $p27^{-/-}$ mice the intermediate lobe is clearly hyperplastic with a nodular appearance resulting in the disruption of the posterior lobe tissue. In both $p27^{-/-}$ mice the anterior lobe cell are compressed relative to the wildtype controls, however, the cell number does not appear to be altered. 45X magnification.

Please replace the paragraph beginning at line 21 of page 10 with the following amended paragraph:

(B) Intermediate lobe cells of $p27^{-/-}$ mice stain positively for POMC-derived peptides. Composite picture of pituitary sections (bottom) stained by avidin-biotin horseradish peroxidase immunocytochemistry for POMC-derived peptides. p, posterior lobe; I, intermediate lobe; a, anterior lobe.

In 11 week old $p27^{-/-}$ (~~left~~ 4B-1) and $p27^{-/-}$ (~~middle~~ 4B-2) mice all intermediate lobe cells are uniformly stained while intermediate lobe cells of 30 week old $p27^{-/-}$ mice (~~right~~ 4B-3) display isolated and heterogeneous staining. 114X magnification.

Please replace the paragraph beginning at line 37 of page 10 and continuing onto page 11 with the following amended paragraph:

(A) The proportion of thymocytes of S-phase is increased in $p27^{-/-}$ mice. Mice were labeled with BrdU for two hours and incorporation into thymocytes was determined by anti-BrdU antibody staining. Sections of thymus isolated from either $p27^{+/+}$ (~~panels a-c~~ FIG. 5A-1 through FIG. 5A-3) or $p27^{-/-}$ (~~panels d-f~~ FIG. 5A-4 through FIG. 5A-6) mice are shown on the left and right respectively. (~~a,d~~ FIG. 5A-1, FIG. 5A-4) 40X magnification of the medullar region. The scale corresponds to 100 microns. (~~b.e~~ FIG. 5A-2, FIG. 5A-5) 20X magnification showing both cortical and medullar regions. (~~e,f~~ FIG. 5A-3, FIG. 5A-6) Same as panels (~~b~~ FIG 5A-2) and (~~e~~ FIG. 5A-5) but anti-BrdU antibody was omitted.

Please replace the paragraph beginning at line 27 of page 11 with the following amended paragraph:

Vaginal smears were taken daily from $p27^{+/+}$ (~~top~~ FIG. 6A through FIG. 6D) or $p27^{-/-}$ (~~bottom~~ FIG. 6E through FIG. 6H) mice and stained with hemotoxylin and eosin. Histology is an indicator of position in estrus (Nelson et al., 1982). $p27^{+/+}$ mice complete estrus in four days (from left to right) passing through diestrus (FIG. 6A), proestrus (FIG. 6B), estrus (FIG. 6C) and metestrus (FIG. 6D), each in a single day. $p27^{-/-}$ mice have a prolonged diestrus and estrus phase, and there is an increase in the amount of mucus in the smears of diestrus phase of the $p27^{-/-}$ mice.

Please replace the paragraph beginning at line 3 of page 12 with the following amended paragraph:

(A) There is a recipricol pattern of staining with p27 and BrdU specific antibodies in the ovaries of $p27^{+/+}$ mice. Ovaries from mice labeled with bromodeoxyuridine were isolated, sectioned, and stained with antibodies against either p27 (FIG. 7A-1), bromodeoxyuridine (FIG. 7A-2) or a rabbit immunoglobulin control antibody (FIG. 7A-3), as indicated below each panel. This antibody was detected with a secondary antibody conjugated to horseradish peroxidase. p27 protein is expressed in luteal cells. Granulosa cell incorporate BrdU.

Please replace the paragraph beginning at line 15 of page 12 with the following amended paragraph:

(B) Ovaries of the $p27^{-/-}$ mice lack the highly differentiated corpus luteum structure observed in controls. Composite picture comparing the ovaries of $p27^{+/+}$ (~~top~~ FIG. 7B-1 and FIG. 7B-2) and $p27^{-/-}$ (~~bottom~~ FIG. 7B-3 and FIG. 7B-4) mice by hemotoxylin and eosin staining using 5 X (FIG. 7B-1 and FIG. 7B-3) and 20 X (FIG. 7B-2 and FIG. 7B-4) magnification ~~(left and right respectively)~~. Mature follicles are present in both animals. CL, corpus luteum; Gr-F, Graffian follicle.

Please replace the paragraph beginning at line 6 of page 27 with the following amended paragraph:

The cyclin/CDK inhibition domain was targeted for gene disruption rather than a complete deletion. To examine if the *p27* mutant allele was capable of expressing any protein fibroblasts were prepared from embryos and analyzed the lysates by immunoblotting (Fig. 1C-1 through Fig. 1C-4). Using an antbody to either full-

length p27 or the carboxyl-terminus of p27 a 20 kDa protein in $p27^{-/-}$ fibroblasts, and a 27 kDa protein in $p27^{+/+}$ fibroblasts were detected. Both proteins were detected in $p27^{+/-}$ fibroblasts (data not shown). In contrast, antibody specific to the amino-terminus of p27 recognized only the 27 kDa protein and not the 20 kDa protein (Fig. 1C-2). The amount of the 20 kDa species and p27 were comparable. Using PCR, RNA transcripts from the p27 mutant allele were amplified and its structure determined; the mutant transcript had the insertion of the neo gene in the antisense direction and normal splicing of the intron separating exons I and II. This transcript contains a predicted open reading frame that encodes amino acid 52-198 of the p27 protein (data not shown).

Please replace the paragraph beginning at line 27 of page 27 with the following amended paragraph:

Taken together, the disruption of the *p27* gene produced an amino-truncated mutant of p27 protein which was called Δ51. To test the function of Δ51 as an inhibitor of CDKs, the His tagged Δ51 from bacteria was purified and added it to extracts of Sf9 cells coinfected with baculoviruses expressing either cyclin E and CDK2, or cyclin D2 and CDK4, and measured the Rb kinase activity of the cyclin/CDK complexes. Δ51 inhibited Rb-phosphorylation by these kinases less efficiently than the full length p27 (Fig. 1D-1 and Fig. 1D-2). Furthermore, Δ51 interacted poorly with cyclin CDK complexes in cells (Fig. 1C-3 and Fig. 1C-4). These results suggest that at the amounts produced in cells, Δ51 will not inhibit G1 CDKs and will not act in a dominant negative fashion to exclude the binding of other CDK inhibitors.

Please replace the paragraph beginning at line 3 of page 30 with the following amended paragraph:

Transgenic mice expressing GH (Palmiter el al., 1982) or IGF-I (Mathews et al., 1988) grow larger than control mice. GH, secreted from the pituitary in response to hypothalamic signals, regulates postnatal body growth mainly by stimulating IGF-I expression in the peripheral tissues. To determine if the size of the *p27*$^{-/-}$ mice was caused by changes of the GH/IGF-I axis the effect of *p27* gene disruption on the pituitary was investigated. All the mice were examined at 11 weeks of age (n=6). The pituitary gland appeared increased in size with a prominent midline intermediate-posterior lobe region. Standard H & E staining revealed intermediate lobe hyperplasia with normal cellular morphology and arrangement, and normal vascularity (Fig. 4A-2 ~~, middle panel~~). The anterior lobes appeared compressed, with increased cellular density, while the posterior lobe appeared normal. In 30 week old mice (n=3) the intermediate lobe was even more hypercellular, with circular nests of cells giving the appearance of nodules (Fig 4B-3 ~~, right panel~~). A marked increase in vascularity was present throughout the lobe, manifested as lakes of distended capillaries filled with red blood cells. Cellular morphology, however, was normal and no evidence of tumor formation was present. The anterior lobes were even more compressed than at 11 weeks. In some animals the tissue mass was sufficiently large as to cause compression of the ventral hypothalamus. The intermediate lobe contains cells that produce alpha-MSH. There was homogeneous staining with an antibody that reacts with POMC-derived peptides, including alpha-MSH, in the IL cells at 11 weeks (Fig. 4B). At 30 weeks, however, the staining was non-homogeneous with some of the nodular regions exhibiting intense staining while in others, staining was markedly decreased, in some, to the point of non-staining.

Please replace the paragraph beginning at line 29 of page 32 with the following amended paragraph:

Thymocyte number is a function of the balance between cell proliferation and cell death. To determine of p27 affected proliferation, death, or both, these processes in the thymocytes of $p27^{-/-}$ mice and controls were examined. To detect thymocytes engaged in S-phase, four week old animals were ~~injecte~~ injected with a single intraperitoneal injection of BrdU, and following dissection two hours later, the extent of BrdU incorporation into chromosomal DNA was measured by immunochemistry. It was found an increase in the number of BrdU-positive thymocytes in thymus from $p27^{-/-}$ animals, in both the cortical region (Fig. 5A-2 and Fig. 5A-5 ~~, panels b and e~~) (largely populated by immature thymocytes) and the medullary region (Fig. 5A-1 and Fig. 5A-4 ~~, panels a and d~~) (largely populated by mature thymocytes). Moreover, this increase in the number of BrdU positive cells was greater than could be accounted for by the increase in cell number. The percentage of BrdU positive cells in random medullar fields of several sections was quantified. This analysis showed that the loss of p27 resulted in high levels of thymocyte proliferation. The mean percentage of BrdU-positive cells in three $p27^{+/+}$ mice was 10% with values in random fields ranging from 3% to 19%. In contrast, the mean percentage of $BrdU^{+}$ cells in three $p27^{-/-}$ mice was 26% with values ranging from 24% to 33%. Furthermore, it was detected increased BrdU incorporation in both the cortical and medullar regions of the thymus suggesting that *p27* gene disruption might affect the percentage of S-phase cells in multiple thymocyte sub-populations. ~~It was also observed increased~~ Increased BrdU incorporation in the spleen was also observed (data not shown).

Please replace the paragraph beginning at line 12 of page 34 with the following amended paragraph:

To determine phases of the estrus cycle, the cytology of vaginal smears was examined. Vaginal smears taken from 8-20 week old mice showed that control mice passed ~~though~~ through diestrus (day 1), proestrus (day 2), estrus (day 3), and metestrus (day 4) in four days (Fig. 6A through Fig. 6D) as described previously (Nelson et al., 1982). In contrast, all the *p27*$^{-/-}$ mice had prolonged estrus cycles, typically showing a prolongation prior to estrus and a delay in exiting estrus (Fig. 6E through Fig. 6H). The most characteristic was the diestrus-proestrus like smear with numerous leukocytes and nucleated epithelial cells within abundant mucous secretion. This smear type often was present for 5-7 days. After a short transition, a prolonged estrus phase followed and persisted for 4-5 days. *p27*$^{-/-}$ female mice (n=8) were capable of mating, albeit infrequently, as confirmed by formation of vaginal plugs; however, none of the mice maintained a pregnancy to the point where swelling of the abdomen could be observed. It was next examined if the mated females conceived. On day 3.5 after formation of the plug three month old females (n=3) were sacrificed and morula stage embryos were isolated from these mice indicating that fertilization had occurred. Furthermore, these morula developed to full-term when transferred to the oviducts of a pseudopregnant normal female. This suggested that ovulation and fertilization do occur in *p27*$^{-/-}$ mice, albeit with decreased frequency, and oocyte development itself does not require p27.

Please replace the paragraph beginning at line 17 of page 35 with the following amended paragraph:

The infertility of *p27*$^{-/-}$ females might be due not only to irregular ovulation, but also to defects in the ability to maintain an environment suitable to maintain

pregnancy. Corpus luteum formation plays an important role in maintenance of pregnancy by actively secreting progesterone and other factors. Granulosa cells, the major somatic cell component of follicles differentiate, by a poorly defined pathway, into progesterone producing luteal cells following ovulation. To determine if p27 was involved in luteal cell differentiation the expression of p27 was examined by immunohistochemistry. In control mice, p27 protein was undetectable in the granulosa cells of the follicle but was abundant in the cells of the corpus luteum (Fig 7A-1 ~~, left panel~~). It was observed a reciprocal pattern of BrdU staining -- incorporation was highest in the follicular granulosa cells and lowest in luteal cells (Fig. 7A-2 ~~, middle panel~~). Examination of $p27^{-/-}$ ovaries indicated a disruption in formation of corpus luteum (Fig. 7B-1 through Fig. 7B-4). These data suggest that the absence of p27 might affect the transition from proliferating granulosa cell to non-proliferating luteal cell.